The Drosophila l(1)zw10 Gene Product, Required for Accurate Mitotic Chromosome Segregation, Is Redistributed at Anaphase Onset

Byron C. Williams,* Tim L. Karr,† John M. Montgomery,* and Michael L. Goldberg*

*Section of Genetics and Development, Biotechnology Building, Cornell University, Ithaca, New York 14853-2703; and†Department of Biochemistry and the Beckman Institute, University of Illinois, Urbana, Illinois 61801

Abstract. Mutations in the gene l(1)zw10 disrupt the accuracy of chromosome segregation in a variety of cell types during the course of Drosophila development. Cytological analysis of mutant larval brain neuroblasts shows very high levels of aneuploid cells. Many anaphase figures are aberrant, the most frequent abnormality being the presence of lagging chromosomes that remain in the vicinity of the metaphase plate when the other chromosomes have migrated toward the spindle poles. Finally, the centromeric connection between sister chromatids in mutant neuroblasts treated with colchicine often appears to be broken with similarly treated control neuroblasts. The 85-kD protein encoded by the l(1)zw10 locus displays a dynamic pattern of localization in the course of the embryonic cell cycle. It is excluded from the nuclei during interphase, but migrates into the nuclear zone during prophase. Metaphase, the zw10 antigen is found in a novel filamentous structure that may be specifically associated with kinetochore microtubules. Upon anaphase onset, there is an extremely rapid redistribution of the zw10 protein to a location at or near the kinetochores of the separating chromosomes.

One strategy to define the molecular components of structures required for accurate chromosome segregation during mitosis is the study of mutations that disrupt this process. This approach has the advantage that it may illuminate important molecules present only in modest concentrations; moreover, the mutant phenotypes may yield useful clues to the function of the corresponding gene products. Several techniques have been developed for the identification of mutations in Drosophila melanogaster that disrupt mitotic chromosome behavior (reviewed in Gatti and Goldberg, 1991). Extensive screening for such mitotic mutants has thus far revealed mutations in only two loci that cause a high percentage of dividing cells to become aneuploid. These genes are rough deal (rod) and lethal on the X chromosome zeste-white 10 (l[zwl0], subsequently abbreviated as zw10; also known as mit[115] [Lindsley and Zimm, 1990]) (Smith et al., 1985; Karess and Glover, 1989; Gatti and Baker, 1989).

In this paper, we report both a detailed analysis of the cytological phenotypes caused by lesions in zw10, as well as molecular studies on the zw10 locus and the protein it encodes. Our results reveal a requirement for zw10 function at a time near anaphase onset for the accuracy of sister chromatid disjunction and/or early anaphase chromatid movement. Such a role for the zw10 product appears to be reflected in the remarkable series of transformations in the intracellular location of this protein we have observed during the cell cycle.

Materials and Methods

Drosophila Stocks

The zw10 alleles zw1051 (Smith et al., 1985; Karess and Glover, 1989; Gatti and Baker, 1989). To obtain larvae hemizygous for mutant zw10 alleles (zw1051), males of genotype zw10°/w° were crossed with C(1)DX.F f/Tr y506 females (from D. Glover, Dundee University, UK). Male larvae, distinguished by the external morphology of the larval gonad, are thus zw10°/Y. Females heterozygous for zw10 mutations and deletions of the zw10 region were generated by crossing zw10°/FM7a females to Df[1]w°/B r w"Y males. Offspring larvae of interest (zw10°/Df[1]w") could be differentiated from their siblings by sex and by virtue of their yellow-colored (w") Malphigian tubules.

Cytology

Metaphase figures were examined using standard cytological procedures.
(Gatti et al., 1974; Gatti and Goldberg, 1991). Ganglia from wandering third instar larvae were dissected in 0.7% NaCl, incubated in 0.5 × 10^{-2} M colchicine in 0.7% NaCl for 1 h at 25°C, and then immersed in 0.5 M sodium citrate (hypotonic solution) for 7 min. The ganglia were then fixed for ~30 s in acetic acid/methanol/distilled water (1:1:2). Fixed brains were immediately transferred into 100% acetic acid in 45% acetic acid on a coverslip and squashed onto a glass slide. For examination of aphrophases, the colchicine and hypotonic fixation were excluded from the above procedure. Cytological preparations according to a different protocol (González et al., 1988; Karess and Glover, 1989) yielded identical results (data not shown). Mitotic index was determined by averaging the number of cells undergoing mitosis under standardized conditions, using a phase-contrast Neofluar 100× oil-immersion Zeiss objective (Carl Zeiss, Inc., Thornwood, NY), 10× oculars, and the Optivar set at 1.25×.

**Nucleic Acids**

The preparation of recombinant DNA from plasmid, cosmid, or bacteriophage lambda vectors, or genomic DNA from *Drosophila* adults, has previously been noted (Gunaratne et al., 1986; Mansukhani et al., 1988b). Genomic clones were isolated either from an EMBL4 genomic library (Gunaratne et al., 1986), or from a cosmid library kindly provided by J. Tamkun (University of Colorado, Boulder, CO). Restriction fragments were subcloned into the polylinker of Bluescript KS- (Stratagene Inc., La Jolla, CA). Transfer of DNA from agarose gels to Gene Screen Plus membranes (New England Nuclear, Boston, MA) was carried out using the alkaline blot method (Sambrook et al., 1989). For radiolabeling, primer extensions from either purified DNA or DNA fragments excised from low-melt agarose gels (SeaPlaque, FMC Marine Colloids, Rockland, ME) were performed essentially as described by Feinberg and Vogelstein (1983). In situ hybridization of DNA fragments labeled with biotin-11-UTP to salivary gland polycysteine chromosomes was performed as previously described (Gunaratne et al., 1986) using the Detek kit (Enzo Biochemistry Inc., New York, NY).

Poly(A)+ RNA was isolated from staged wild-type *Drosophila* according to the procedure of Dombrádi et al. (1989). Electrophoresis of glyoxalated poly(A)+ RNA on agarose gels, transfer to Hybond-N membranes, and hybridization of Northern blots with labeled probes was also carried out as detailed by the same authors.

Full-length *zw10* cDNA clones were isolated from a *Drosophila* imaginal disc cDNA library supplied by Dr. Nicholas Brown (Harvard University, Cambridge, MA). Since the pB40 vector used allows directional cDNA cloning (Brown and Kafatos, 1988), the orientation of transcription could be readily determined by restriction analysis. A BglII-Not fragment containing the entire *zw10* cDNA was cloned into the BamHI and NotI sites of Bluescript KS−, yielding the construct 20.4KS−. Deletions were constructed using partial EcoIII nuclease digestion as described by Henikoff (1984) using the Erase-a-Base kit (Promega Corp., Madison, WI). 20.4KS− was digested with *KpnI* and *ClaI* for deletions starting from the 5'-end of the cDNA, while *SacI* and *NotI* were used for deletions starting from the 3'-end. The resulting *SacI* and *NotI* fragments were isolated and the ends were filled in with Klenow fragment to create blunt termini. A 1.5 kb *SacI* fragment of the *zw10* cDNA was blunt-end ligated into the BamHI site of pATH3 (which was made blunt by filling in with Klenow DNA polymerase), so that *zw10* was in frame with *trpE* sequences. The resulting construct was called pATH3-*zw10*. Derivatives containing shorter *zw10*-specific segments were created by digesting pATH3-*zw10* with *XhoI* or *HindIII* and recircularizing the products in a very dilute ligation mixture. The *XhoI* derivative (pATH3-*zw10X*) lacks the central portion of *zw10* (amino acids 177-445) and the *HindIII* derivative (pATH3-*zw10H*) does not contain the COOH-terminal end (amino acids 528-721). It should be noted that all *lacZ-*zw10 and trpE-*zw10* fusion proteins lack the NH2 terminus of the *zw10* protein (amino acids 1-76).

**Germline Transformation**

A 4.6 kb BamHI fragment from the cosmids genomic clone cosB was inserted into the BamHI site of pNW, a transformation vector carrying a mini- white gene (Klenczm et al., 1987). The resulting construct was injected into w; Sb e Delta2-3/Tr6, Ubx embryos, which express transposable (Robertson et al., 1988); GO survivors were single-pair mated to *w* males and the GI was screened for pigment eye color. Independently derived transformants were isolated and mated to *w* males to determine the linkage of the marker to *w* and to one another. In one line (1-1) the fragment had integrated into the Delta2-3 and Sb. In two others (2A and 3A), *w* was linked to Ubx, a marker on TM6. Males containing these autosomaly located transduced DNA fragments were mated with *w101* Balmers females to test for the presence of non-Bar eyed *zw10* male progeny in the next generation. Three different *zw10* alleles (*zw10D*, *zw10D2*, and *zw10D2*2) were rescued by the *1-1*, 2A, and 3A transformant lines. In each case, the low viability, sterility, eye morphology, and mitotic (cytological) phenotypes of the *zw10* mutatations were complemented by the autosomal fragment.

**Antibody Production and Purification**

A 2.2 kb NruI-Not fragment from the full length *zw10* cDNA was blunt-end ligated into frame with *trpE* sequences (Dieckmann et al., 1985). Independently derived *zw10* cDNA-NruI-Not fragment used to make lacZ-*zw10* was blunt-end ligated into the BamHI site of pATH3 (which was made blunt by filling in with Klenow DNA polymerase), so that *zw10* was in frame with *trpE* sequences. The resulting construct was called pATH3-*zw10*. Derivatives containing shorter *zw10*-specific segments were created by digesting pATH3-*zw10* with *XhoI* or *HindIII* and recircularizing the products in a very dilute ligation mixture. The *XhoI* derivative (pATH3-*zw10X*) lacks the central portion of *zw10* (amino acids 177-445) and the *HindIII* derivative (pATH3-*zw10H*) does not contain the COOH-terminal end (amino acids 528-721). It should be noted that all *lucZ-*zw10 and trpE-*zw10* fusion proteins lack the NH2 terminus of the *zw10* protein (amino acids 1-76).

**Immunofluorescence**

Wild-type Oregon R embryos were collected for 2 h to enrich for the presence of synctial blastoderm embryos. Embryos were washed, dechorionated, and fixed with formaldehyde in the presence of taxol as described.
Results

Cytological Characterization of Mutant Larval Brains

The viability of flies homozygous or hemizygous for mutant alleles of zwl0 is severely reduced. The majority of zwl0 mutant animals die during late larval and pupal stages (Shannon et al., 1972), survival through embryonic and early larval stages is thought to be ensured by maternal wild-type gene product from heterozygous mothers (Gatti and Baker, 1989; see also Discussion below). Division of brain neuroblasts and imaginal disk cells occurs during the third larval instar, and is required for subsequent adult metamorphosis but not for larval life itself. The third instar larvae of mitotic mutants thus contain populations of cells undergoing improper mitosis. In particular, the dividing cells in the neural ganglia (brain neuroblasts) of third instar larvae are well suited to cytological observation which may suggest the origin of the mitotic defect. To elucidate the role played by the zwl0 product in ensuring proper chromosome segregation during cell division, we have continued the analysis of mitosis in zwl0 mutant larval neuroblasts initiated by Smith et al. (1985).

Aneuploidy. In preparations of wild-type larval ganglia, four pairs of chromosomes are clearly visible in each metaphase figure (Fig. 1 a). However, a high proportion (\(\sim 50\%\)) of brain cells in animals homozygous or hemizygous for a variety of zwl0 alleles are hyperploid (Table I; Smith et al., 1985). This is almost certainly an underestimate of the number of cells with abnormal chromosome complements: because chromosomes may be lost during squashing, hypoploid nuclei have not been scored in these studies. Examples of hyperploid mitotic figures are shown in Fig. 1, b and c. The distribution of karyotypes (Table I) suggests that zwl0 mutations cause essentially random mitotic segregation of chromosomes, with all major chromosomes affected to a similar extent.

Abnormal Anaphases. Aneuploidy caused by zwl0 mutations clearly involves improper chromosome segregation at anaphase. In wild-type male larval ganglia, most anaphases resemble that shown in Fig. 1 d; only a small percentage of anaphases can be classified as aberrant. Conversely, a high frequency (\(\sim 40\%\)) of abnormal anaphases are present in neuroblasts of animals hemizygous for any of several zwl0 mutant alleles (Table II). The defect most often observed is the presence of one or more chromatids lagging at the metaphase plate when the remainder have already migrated to their respective poles (Fig. 1, e and f). In some cases, some chromatids are clearly pulled at their kinetochore but are nonetheless delayed in their approach to a pole, while other chromatids appear not to be subjected to poleward forces at the time of fixation (Fig. 1 g). It is of interest that lagging chromatids are often found in the near vicinity of their sisters, which thus appear to be similarly affected (Fig. 1, f and g). In a fraction of cases, anaphases are either completely disorganized (Fig. 1 i), or result in obviously unequal chromosome complements at the two spindle poles (Fig. 1 h). Any of these aberrant events at anaphase could potentially produce aneuploidy in daughter cells.

Precocious Sister Chromatid Separation. Smith et al. (1985) have suggested an alternative explanation for the high

---

1. Abbreviations used in this paper: KMT, kinetochore microtubule; MT, microtubule; PSCS, precocious sister chromatid separation; TBST, TBS + Triton; TTBS, Tween TBS.
Figure 1. Cytological effects of zwl0 mutations in neuroblast cells. (a–c) Colchicine-treated metaphase figures. (a) Oregon R wild-type moles showing two pairs of metacentric autosomes, a telocentric X chromosome, a linear, heterochromatic Y chromosome (4A, XY), and two dot-like fourth chromosomes not scored in subsequent figures. (b) Adjacent aneuploid zwl0St/Y cells (4A, XXY and 6A, XY). (c) 7A, 4XY zwl0St/Y cell. (d–i) Anaphase figures from cells not treated with colchicine. (d) Oregon R wild type. (e–f) zwl0St/Y Arrows denote lagging chromatid(s). (g) zwl0St/Y The open arrow shows a lagging sister chromatid pair; the black arrow indicates a chromatid apparently not subjected to poleward forces. (h) zwl0St/Y Unequal anaphase. (j–l) Colchicine-treated metaphase figures showing precocious sister chromatid separation in zwl0St/Y (j and l) and zwl0St/Y (k) neuroblasts. In some cases, the apposition between sister chromatids, particularly at centromeric regions, remains close (l; arrow in k).

levels of aneuploidy observed in zwl0 mutant brain cells. They reported that in many colchicine-induced metaphase figures in mutant ganglia, sister chromatids appeared to be physically unconnected with each other. These investigators proposed that prematurely separated chromatids could establish connections at random to microtubules (MTs) emanating from the two spindle poles, and would thus segregate randomly at anaphase.

We have verified the occurrence of this form of precocious sister chromatid separation (PSCS). In the presence of col-
Table I. Aneuploidy in l(l)zw10 Mutant Brains

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of brains</th>
<th>Number of figures</th>
<th>4AXY</th>
<th>5AXY</th>
<th>4AXXY</th>
<th>4AXYY</th>
<th>6AXY</th>
<th>5AXXY</th>
<th>5AXYY</th>
<th>7AXY</th>
<th>Other</th>
<th>Percent aneuploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>zw10gr2/Y</td>
<td>6</td>
<td>240</td>
<td>95</td>
<td>41</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>55</td>
<td>60.4</td>
</tr>
<tr>
<td>zw10grm/Y</td>
<td>7</td>
<td>232</td>
<td>104</td>
<td>36</td>
<td>18</td>
<td>11</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>46</td>
<td>55.1</td>
</tr>
<tr>
<td>zw10p/Y</td>
<td>6</td>
<td>323</td>
<td>147</td>
<td>53</td>
<td>24</td>
<td>19</td>
<td>9</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>59</td>
<td>54.5</td>
</tr>
<tr>
<td>X/Y (Oregon R)</td>
<td>10</td>
<td>1000</td>
<td>989</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Neural ganglia from males hemizygous for the indicated zw10 alleles or from wild-type Oregon-R males were dissected, incubated with colchicine, treated with hypotonic solution, fixed, stained, and squashed as described in Materials and Methods. *Number of brains and Number of figures* refer respectively to the total number of brains and the total number of colchicine-induced metaphase figures examined. The number of figures with particular karyotypes are listed. Because of difficulties in recognition of the small fourth chromosome, only the large second and third autosomes (A), which cannot be distinguished from each other, and the X and Y chromosomes were scored. Thus, the normal diploid male karyotype would be 4AXY. Similar results were obtained by Smith et al. (1985) for brains from individuals hemizygous for other l(l)zw10 alleles.

Table II. Mitotic Parameters of l(1)zw10 Mutant Brains

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Percent aberrant anaphases</th>
<th>PSCS</th>
<th>MI</th>
<th>Percent anaphase</th>
</tr>
</thead>
<tbody>
<tr>
<td>zw10gr2/Y</td>
<td>40.3 (149:9)</td>
<td>28.3 (956:6)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>zw10grm/Y</td>
<td>38.6 (153:8)</td>
<td>40.4 (755:5)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>zw10p/Y</td>
<td>47.4 (156:9)</td>
<td>30.0 (113:6)</td>
<td>0.60</td>
<td>15.4 (2305:7)</td>
</tr>
<tr>
<td>C(l)DX,yf/Y</td>
<td>ND</td>
<td>1.1 (3000:15)</td>
<td>0.55</td>
<td>14.1 (2380:9)</td>
</tr>
<tr>
<td>X/Y (Oregon R)</td>
<td>3.2 (222:10)</td>
<td>1.8 (1000:5)</td>
<td>0.57</td>
<td>14.4 (3211:8)</td>
</tr>
<tr>
<td>zw10p/Df(l)w271</td>
<td>40.7 (22:2)</td>
<td>32.3 (295:2)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

This table catalogs the results of three separate experiments. (a) The percentage of anaphases which appear abnormal is scored under *Percent aberrant anaphases*. This information was obtained from neural ganglia that were fixed and stained in the absence of colchicine or hypotonic treatments (see Materials and Methods). The first of the adjacent numbers, presented in smaller type and within parentheses, indicates the total number of anaphascs scored; the second number refers to the number of brains examined. (b) *PSCS* shows the percentage of colchicine-induced, hypotonic-treated metaphase figures in which the centromeric connection between one or more pairs of sister chromatids appears to be severed. The larger of the adjacent numbers within parentheses indicates the total number of metaphase figures, the smaller, the total number of brains. (c) MI is expressed as the number of nuclei in division per optic field under standard conditions (see Materials and Methods). MI is not clear whether or not the connection at the centromere is broken. A stronger argument is that random attachment of the chromatids to the spindle should not result in the lagging chromosomes or chromatids observed in anaphase figures (although figures with unequal numbers of chromosomes at the two poles would be apparent). We thus consider that PSCS is possibly an artefactual colchicine-dependent effect that may nonetheless reveal important differences between the centromeres of chromosomes in wild-type and mutant cells (see Discussion).

Other Mitotic Parameters. It is conceivable that the cytological effects of zw10 mutations result from difficulties in progression through the cell cycle. For example, lagging chromatids appear at high frequencies after release of cells from a metaphase-arrested state (Hsu and Satya-Prakash, 1985). We have thus measured two parameters that provide some indication of the numbers of cells in various stages of mitosis. The mitotic index, an average of the number of cells undergoing mitosis per optic field, is similar in wild-type larval brains and in larval brains from a mutant that appears to represent the null state of the zw10 locus (see below; Table II). In addition, the ratio between the number of cells in anaphase relative to the total number of mitotic figures is also not grossly affected by this mutation in zw10 (Table II).

Tests for the Null Phenotype. To determine whether the phenotypes discussed above mirror the null state of the zw10 gene, we have examined the cytological characteristics of the chromatin of the mutant nuclei display one or more chromatid pairs with PSCS (Table II). Sister chromatids are often adjacent to each other, yet appear unattached at their centromeres (Fig. 1, j, k, and l). Control ganglia from C(l)DX, yf/Y siblings of hemizygous mutants show PSCS at a frequency of <2%. Particular care was taken to ensure similar treatment of mutant and control brains. Both types of ganglia were dissected at the same time from animals grown in the same bottle; the brains were mixed and exposed to colchicine in the same vessel. Mutant and control brains were squashed together on the same slide; control ganglia can be recognized by the characteristic morphology of the attached X chromosome (see Materials and Methods).

The absence of sister chromatid separation in wild-type, colchicine-treated mutant nuclei display one or more chromatid pairs with PSCS (Table II). Sister chromatids in these neuroblasts remain show PSCS. Sister chromatids in these neuroblasts remain closely apposed to each other (data not shown); however, it is not clear whether or not the connection at the centromere is broken. A stronger argument is that random attachment of the chromatids to the spindle should not result in the lagging chromosomes or chromatids observed in anaphase figures (although figures with unequal numbers of chromosomes at the two poles would be apparent). We thus consider that PSCS is possibly an artefactual colchicine-dependent effect that may nonetheless reveal important differences between the centromeres of chromosomes in wild-type and mutant cells (see Discussion).

Other Mitotic Parameters. It is conceivable that the cytological effects of zw10 mutations result from difficulties in progression through the cell cycle. For example, lagging chromatids appear at high frequencies after release of cells from a metaphase-arrested state (Hsu and Satya-Prakash, 1985). We have thus measured two parameters that provide some indication of the numbers of cells in various stages of mitosis. The mitotic index, an average of the number of cells undergoing mitosis per optic field, is similar in wild-type larval brains and in larval brains from a mutant that appears to represent the null state of the zw10 locus (see below; Table II). In addition, the ratio between the number of cells in anaphase relative to the total number of mitotic figures is also not grossly affected by this mutation in zw10 (Table II).

Tests for the Null Phenotype. To determine whether the phenotypes discussed above mirror the null state of the zw10 gene, we have examined the cytological characteristics of the chromatin of the mutant nuclei display one or more chromatid pairs with PSCS (Table II). Sister chromatids in these neuroblasts remain show PSCS. Sister chromatids in these neuroblasts remain closely apposed to each other (data not shown); however, it is not clear whether or not the connection at the centromere is broken. A stronger argument is that random attachment of the chromatids to the spindle should not result in the lagging chromosomes or chromatids observed in anaphase figures (although figures with unequal numbers of chromosomes at the two poles would be apparent). We thus consider that PSCS is possibly an artefactual colchicine-dependent effect that may nonetheless reveal important differences between the centromeres of chromosomes in wild-type and mutant cells (see Discussion).

Other Mitotic Parameters. It is conceivable that the cytological effects of zw10 mutations result from difficulties in progression through the cell cycle. For example, lagging chromatids appear at high frequencies after release of cells from a metaphase-arrested state (Hsu and Satya-Prakash, 1985). We have thus measured two parameters that provide some indication of the numbers of cells in various stages of mitosis. The mitotic index, an average of the number of cells undergoing mitosis per optic field, is similar in wild-type larval brains and in larval brains from a mutant that appears to represent the null state of the zw10 locus (see below; Table II). In addition, the ratio between the number of cells in anaphase relative to the total number of mitotic figures is also not grossly affected by this mutation in zw10 (Table II).

Tests for the Null Phenotype. To determine whether the phenotypes discussed above mirror the null state of the zw10 gene, we have examined the cytological characteristics of
larvae heterozygous for a deletion (Df(1)w⁶⁷f) that removes zwl0 and for a zwl0 mutant allele. Levels of hyperploidy (52.1% [74/142 nuclei] for zwl0⁶⁷f/Df(1)w⁶⁷f and 49.6% [64/129] for zwl0⁶⁷f/Df(1)w⁶⁷f) were similar to values observed in zwl0 hemizygous animals (compare with Table I). Both frequencies of aberrant anaphases and PSCS in zwl0⁶⁷f/Df(1)w⁶⁷f mitotic figures were also close to values seen for the mutant alleles alone (Table II). If the zwl0 alleles examined were hypomorphic, it would be expected that deficiency/mutant heterozygotes would display a stronger phenotype than animals homozygous or hemizygous for the mutation alone. These classical genetic tests therefore suggest that the mutations we have analyzed, all of which have similar effects, characterize the null state of the zwl0 gene; this hypothesis is supported by analysis of the zwl0 protein in mutant animals, as reported below.

**Molecular Mapping of the zwl0 Locus**

A portion of the *Drosophila* X chromosome including the zwl0 gene was cloned during the course of a chromosomal walk through the *zeste-white* interval (Goldberg et al., 1983; Gunaratne et al., 1986; our own unpublished data). Within this region, zwl0 must be located distal (relative to the centromere) of the deletion Df(1)64j4, and completely within the duplication Dp(1;2)w⁷⁰k¹³ (Fig. 2). The locations of the breakpoints associated with these two rearrangements were ascertained by whole genomic Southern blots and by in situ hybridization to salivary gland polytene chromosomes from larvae of the appropriate genotype (Fig. 2; data not shown). These results delimit zwl0 to a region of 11 kb within the cloned X chromosome interval.

Two observations suggested that part of the zwl0 locus must lie near the Dp(1;2)w⁷⁰k¹³ breakpoint at coordinate −0.3 on Fig. 2. First, the genetic map distance between zwl0 and the adjacent lethal complementation group zw4 is very small (<0.026 map units, or <7.8 kb based on a conversion of 1 map unit = 300 kb in this region of the X chromosome) (Kidd et al., 1983), yet Dp(1;2)w⁷⁰k¹³ encompasses zwl0 but not zw4. Second, a spontaneous allele, zwl0 s¹ (Schalet, 1986), is associated with a 4.5-kb insertion of DNA at coordinate +1.7. This DNA insertion in zwl0 s¹ is most likely a Doc mobile element, based on the similarity of their restriction maps (Driver et al., 1989).

Northern blot analysis of adult poly(A)⁺ mRNA re-

---

**Figure 2.** Genetic and molecular map of the zwl0 region. Lethal complementation groups in the vicinity of l(1)zwl0 are shown at the top of the figure, distances between genes are given in centimorgans (Judd et al., 1972). (a) Loci contained within the duplication Dp(1;2)w⁷⁰k¹³; genes deleted in Df(1)64j4 are indicated by the hatched bar. The molecular map is shown only for a 16-kb region immediately adjacent to the zwl0 gene; coordinates are given in kb, with 0 defined as the BamHI site at the left end of Fragment A used to rescue zwl0 phenotypes (see text). The mobile element in zwl0 s¹ is diagrammed to the same scale as the remainder of the restriction map. The direction of transcription producing poly(A)⁺ RNAs from this region is shown by arrows; the sizes of mature transcripts are indicated below. H, HindIII; X, XhoI; B, BamHI; R, EcoRI; C, SacI; N, NotI; and S, SalI.
Dp(l;2)w § encoding the entire 2.6-kb transcript but containing no other candidate for the zw10 mRNA. A 4.8-kb fragment of genomic DNA (Fragment A: coordinates 0--4.8 on Fig. 2), encoding the entire 2.6-kb transcript but containing no other complete transcriptional unit, was transformed into *Drosophila* by P element-mediated germline transformation. This fragment, as present in three independent autosomal transformant lines, is necessary and sufficient to rescue the zw10 lethal phenotype of several zw10 alleles (Materials and Methods). Fragment A also repairs the zw10 mitotic phenotype. For example, in rescued zw10 § P(§A;Fragment A)/+ larval ganglia, the frequency of aneuploidy (1.2%) and PSCS (4.3%) were at wild type levels (compare with Table II), while anaphases were normal (not shown). Thus, the entire zw10 locus must reside within the 4.8-kb Fragment A depicted in Fig. 2, and appears to be transcribed into a poly(A)+ RNA 2.6 kb in length.

**Molecular Analysis of the zw10 Locus**

The zw10 transcript is developmentally regulated. The highest levels of this RNA are found in embryos and in adult females (Fig. 3), consistent with the idea that maternally supplied zw10 supports the rapid syncytial divisions of early embryogenesis. Levels of zw10 RNA are substantially decreased during the first and second larval instar, but then increase in third instar larvae and in early pupae. Presumably, this reflects the increased number of proliferating cells in late larval/early pupal imaginal discs. Only a single 2.6-kb RNA band is seen at any stage of development on Northern blots; it nonetheless remains possible that alternatively processed species of similar length, or rare RNAs of different sizes, may be produced from the same transcriptional unit.

Three cDNA clones homologous to the 2.6-kb zw10 mRNA were isolated from an imaginal disc cDNA library (Brown and Kafatos, 1988). Substantial overlap in the restriction maps of these cDNAs suggests that they all represent the same species (data not shown). The sequence of the longest of these cDNAs (2,576 bp) contains an open reading frame beginning at nucleotide 103 that encodes a protein of 721 amino acids (Fig. 4). However, it should be cautioned that the nucleotides in the vicinity of this putative initiation codon show only partial agreement with the Drosophila translation start consensus (CA/G/A/C/A AUG; Cavener and Ray, 1991). Better matches are seen in the vicinity of the downstream methionine codons at nucleotide positions 232--235, 343--345, and 400--402. Although it is thus possible that translation of the corresponding mRNA may initiate at any of these positions, the size of the zw10 protein (see below) is most consistent with use of the codon indicated in Fig. 4.

A computerized search has failed to reveal significant homologies to any protein within the Genbank, EMBL, PIR, and Swiss-Prot databases. Given the cell cycle-dependent changes in the intracellular location of zw10 protein that will be described below, it is of interest that the zw10 sequence contains motifs that may be subject to phosphorylation. A potential tyrosine kinase phosphorylation site (consensus sequence R/K[2,3]D/E[2,3]Y) is found beginning at amino acid 48, while sites possibly available for phosphorylation by the cdc2 kinase (TP and SP) start at amino acids 63 and 167 (Shalloway and Shenoy, 1991).

**Antibodies Against the zw10 Protein**

Polyclonal rabbit antibodies were generated against gel-purified β-galactosidase-zw10 fusion protein (β-gal-zw10) that had been produced in *E. coli* cells. Crude antisera were purified by immunoaffinity chromatography against trpE-zw10 protein fusions (see Materials and Methods for further information concerning the preparation and characterization of these reagents). The purified antibodies recognize a single band of 85 kD, consistent with the size of the predicted zw10 protein, in Western blots of 0--16-h old embryos, of third instar larvae, and of *Drosophila* Schneider Line 2 tissue culture cells (Fig. 5, A and B). When the same Schneider cell line is transfected with a construct containing the complete zw10 open reading frame under the control of the strong T7 promoter, a 721 amino acid-long protein that will span the site of the zw10 breakpoint and that spanned the site of the zw10 insertion element (Fig. 2); this was therefore a likely candidate for the zw10 mRNA. A 4.8-kb fragment of genomic DNA (Fragment A: coordinates 0--4.8 on Fig. 2), encoding the entire 2.6-kb transcript but containing no other complete transcriptional unit, was transformed into *Drosophila* by P element-mediated germline transformation.
Figure 4. Sequence of the zwi0 cDNA. The nucleotide sequence of the coding strand of the longest zwi0 cDNA clone, with position 1 assigned to the 5'-most base. The ATG at 103 is the most likely site of initiation, but other ATGs (at 232, 343, and 400) are also possible choices (see text). A stop codon (TAG) at position 2,268 has been signified by an asterisk. The sequence is thus postulated to contain a 5'-untranslated region from nucleotides 1-102, and a 3'-untranslated region from nucleotides 2,269-2,576. A single long open reading frame encodes a predicted protein sequence, here represented by the single-letter code, of 721 amino acids in length, having a molecular weight of 82,115. These sequence data are available from EMBL/GenBank/DDBJ under accession number X64390.

actin5C promoter (Bond and Davidson, 1986), this same band is clearly overproduced (Fig. 5 A). Evidence that the antibody reacts specifically with the zwi0 gene product is provided by the analysis of extracts from larvae hemizygous for mutant zwi0 alleles. The zw1051 and zw1012 mutants have no detectable 85-kd zwi0 protein (Fig. 5 B). The zw1051 allele does not appear to encode stable zwi0 cross-reactive polypeptides, and is thus likely to represent the null state of the locus. This result is expected, because the zw1051 gene is interrupted by a DNA insertion (Fig. 2). These exogenous sequences are located within an exon near the middle of the coding sequence (see Materials and Methods), between nucleotides 1,202 and 1,375 (Fig. 4). Males of genotype zw10022/Y display a band of ~75 kD that we presume to be a truncated product caused by a nonsense or frameshift mutation; the amount of this 75-kD protein is reduced relative to the amount of 85-kD zwi0 protein in wild type (Fig. 5 B).

Immunolocalization of the zw10 Antigen
To determine the location of zw10 protein at different stages of the cell cycle, we chose to examine mitosis in syncytial blastoderm embryos by indirect immunofluorescence using...
the purified antibodies described above. Distinctive patterns are observed at different stages of mitosis. The progression of these zw10 staining patterns through the cell cycle is also identical in the mitotic domains of cultured embryos (Foe, 1989) and in larval neuroblast cells, so the observed distribution of zw10 protein is not specific to syncytial blastoderm stages 9–13 (data not shown).

Prometaphase to Metaphase. During interphase and most of prophase, the zw10 protein appears to be excluded from the nucleus (see below). At a time we assume corresponds to the partial breakdown of the nuclear envelope at the beginning of prometaphase (Stafstrom and Staehelin, 1984; Hiraoka et al., 1990), zw10 antigen starts to coalesce in the nuclear domain (Fig. 6, a and d). The staining is mostly amorphous and surrounds the condensing chromosomes, but a few discrete spots appear to be recognized by the antibody.

By metaphase, zw10 protein becomes localized to discrete, filamentous structures residing in the central, longitudinal portion of the spindle (Fig. 6, b and e; Fig. 7, A–C). The strands originate near the centrosomes at opposite poles of the spindle apparatus, but zw10 staining of the centrosomes per se is not observed (Fig. 7 B). Remarkably, and in contrast with the total tubulin pattern, zw10 antigen appears to pass directly through the chromosomal mass at the metaphase plate (Fig. 7, A and C).

Anaphase. At the beginning of anaphase, zw10 antigen becomes excluded from the region of the metaphase plate, and the filaments shorten from their ends nearest the centrosomes. As a result, zw10 protein becomes concentrated into punctate structures at the leading edges of the separated chromatids (Fig. 6, c and f; Fig. 7, D–F). At this level of resolution, the number and position of these structures is consistent with localization at the centromere/kinetochore region of individual chromatids. The zw10 protein appears to remain at or near the kinetochores through the remainder of anaphase, although the shortening of kinetochore microtubules (KMTs) at these later stages of anaphase renders resolution in the kinetochore–centromere interval difficult (Fig. 6, g and j).

The transition between the metaphase and early anaphase states of zw10 is very rapid. Mitosis in syncytial Drosophila embryos is metasynchronous: division starts at successively later times in nuclei increasingly closer to the embryonic equator, forming a mitotic wave (Foe and Alberts, 1983). As seen in Fig 8, adjacent nuclei can display the mature metaphase and anaphase zw10 patterns, but intermediate structures are sometimes observed. Although the rate of mitotic wave propagation is quite variable, at an average value of 100 µm/min (Foe and Alberts, 1983), and given the approximate distance between adjacent nuclei (~20 µm at blastoderm stages 11–12), we estimate that the transition between the metaphase and anaphase states of zw10 may be accomplished in periods as short as 10–12 s.

Telophase, Interphase, and Prophase. At the beginning of telophase, the zw10 antigen becomes excluded from the domain of the reforming nucleus (Fig. 6, h and k). Staining is also restricted to the extranuclear cytoplasm during interphase (Fig. 6, i and l) and prophase (not shown).

Discussion

zw10 Mutations Affect Chromosome Segregation

Several observations indicate that zw10+ function is necessary to ensure accurate chromosome segregation during cell division in most, if not all, Drosophila tissues. (a) A temperature-sensitive mutation of zw10 (zw10o) caused a 120-fold increase in the incidence of clones of homozygous multiple wing hair (mwh) cells in the wings of zw10o/Y; mwh/+ males raised at semi-restrictive temperature. Additional tests implicated an elevated frequency of mitotic non-
Figure 6. Immunolocalization of the zwiO protein during the cell cycle in wild-type syncytial blastoderm embryos. Embryos were fixed, stained, and processed for indirect immunofluorescence as described (see Materials and Methods). (a–c and g–i) zwiO protein localization is shown; below each of these is shown the corresponding DNA staining (d–f and j–l). (a and c) Prometaphase; zwiO protein moves into the nuclear domain; punctate staining is visible. (b and e) Metaphase; zwiO filamentous strands are completely formed; considerable substructure is apparent. (c and f) Anaphase; zwiO protein is rapidly relocalized to the kinetochore regions of separating sister chromatids (arrows). (g and j) Late anaphase; zwiO protein remains on kinetochores (arrows). (h and k) Telophase; zwiO antigen disappears from kinetochores. Faint cytoplasmic staining is apparent. (l and i) Interphase; no nuclear localization is visible. Bar, 10 μm.

disjunction in the formation of these somatic clones (Smith et al., 1985). (b) As discussed above, a high proportion of zwiO mutant larval brain neuroblast cells are aneuploid. Many anaphase figures in these cells are obviously aberrant. (c) Upon completion of the second meiotic division in escaper males, spermatid nuclei containing different numbers of chromosomes are produced (our own unpublished observations). It is not presently clear whether missegregation occurs during the first or second meiotic divisions, or during earlier mitoses in the male germline. (d) Maternally supplied zwiO gene product is necessary for embryogenesis, as shown by germline clonal analysis (Perrimon et al.,...
Figure 7. Localization of zw10 protein in comparison with chromosomes, centrosomes, and tubulin. The embryos were processed for indirect immunofluorescence and confocal microscopy as outlined in the Materials and Methods. Superimposed images were obtained from the same focal plane. (A) At metaphase, zw10 protein (orange) filaments extend through the chromosomal mass (blue) and have considerable substructure (particularly apparent in the nucleus at far left). (B) Centrosomes (yellow) and the zw10 protein (red) at metaphase. zw10 protein filaments extend to, but do not overlap with, centrosomes. (C) Tubulin staining (green) and zw10 protein (red) at metaphase. The zw10 protein is localized to only a subsection of the mitotic spindle (arrow; regions of overlap between zw10 and tubulin are yellow.) (D) Regions in or near kinetochores of chromosomes (blue) are the sites of zw10 protein localization (red) during early-mid anaphase. (E) At early anaphase, centrosomes (yellow; solid arrows) are well resolved from zw10 (red; outline arrow). (F) zw10 (red) is restricted to discrete spots at the centrosome-distal portion of each hemispindle at anaphase onset (tubulin staining is green while overlap is yellow). Thus, zw10 is not found along the length of the KMTs at this stage of the cell cycle. Bars, 10 μm.

1989). In addition, syncytial blastoderm embryos derived from zw10 escaper females (see below) have abnormally spaced nuclei of aberrant morphology (our own unpublished results).

These findings may be understood in terms of the varying requirements for cell division at different stages in Drosophila development. Maternally derived gene products within the egg must be used to construct the molecular machinery required for the rapid embryonic mitoses after fertilization, because little transcriptional activity occurs during this period. For many proteins involved in mitosis, the maternal contribution is sufficient to allow development into larval
Cytological Effects of zw10 Mutations

The evidence presented above clearly shows that mutations in the zw10 gene cause aberrant anaphases within larval neuroblasts, in turn generating a high proportion of aneuploid brain cells. We have also verified that the phenomenon of PSCS is an additional consequence of zw10 lesions in colchicine-treated neural ganglia. However, the proposition that the anaphase defects observed are due to PSCS before anaphase onset remains questionable, because of uncertainties about the state of cells exposed to colchicine and because of our lack of knowledge concerning the forces that determine sister chromatid interactions.

The consideration of published precedents for the cytological phenotypes discussed above may be instructive in speculations concerning potential functions of the zw10 protein. Laggard sister chromatids have been postulated to result from damage to the centromere/kinetochore produced by drugs (Brinkley et al., 1985; Hsu and Satya-Prakash, 1985), by injection with anticientromere antibodies (Bernat et al., 1991), or by microirradiation of kinetochores with a laser (McNeill and Berns, 1981). PSCS has been documented in two Drosophila meiotic mutants, orientation disruptor (ord) (Goldstein, 1980; Lin and Church, 1982) and mei-S332 (Davis, 1971; Kerrebrock et al., 1992). Finally, several cytological features of zw10 mutations, including aneuploidy and lagging chromatids at anaphase, have been noted both in Drosophila mutant for the gene rough deal (rod) (Karess and Glover, 1989) and in cell cultures derived from patients with the human genetic disorder Roberts Syndrome (Jabs et al., 1991).

A puzzling phenotypic consequence of mutations in zw10 arises from the survival of a small number of "escaper" adults of both sexes that are either hemizygous or homozygous for all known zw10 mutations. Both male and female escapers are sterile and exhibit a variety of cuticular defects (Shannon et al., 1972). Although the sterility of zw10 escaper females can be explained by embryonic mitotic defects we have observed in their progeny, the fact that mutant hemizygous males contain immotile sperm (Shannon et al., 1972; our own unpublished observations) is more difficult to understand. Examination of the "onion stage" of spermatogenesis in male testes shows the presence of variable-sized spermatid nuclei, indicating that chromosomal nondisjunction or chromosome loss has occurred in the male germline. However, this alone does not account for sperm immotility: even sperm that contain only the tiny fourth chromosome are motile and capable of fertilization (Lindsley and Grell, 1969). The zw10 product might therefore play an additional role in spermatogenesis or sperm function that is independent of chromosome segregation.

zw10 Protein Is Dynamically Localized in Mitotic Structures

Embryonic zw10 protein undergoes cell-cycle dependent redistribution to different components of the mitotic apparatus. At the prophase-prometaphase transition, zw10 protein becomes localized in the nuclear domain, and becomes associated with, or forms, a filamentous structure which persists through metaphase. A very rapid transition to a region at or near kinetochores is seen coincident with anaphase onset. At telophase, the zw10 protein is excluded from the reforming nuclear domain and becomes dispersed in the cytoplasm. The dynamic nature of zw10 distribution through these cell cycles is most likely because of intracellular movement of the same protein pool, rather than to reflect new protein synthesis, given the rapidity of embryonic nuclear divisions (Foe and Alberts, 1983).

The nature of the structures recognized by anti-zw10 antibodies at metaphase and anaphase is uncertain. It is possible that the zw10 protein filaments form an independent structure not directly associated with MTs. Alternatively, some manner of zw10 association with spindle MTs would seem possible. It is clear that the zw10 antigen could not be associated
with all MTs within the spindle, but is instead generally confined to a narrower region than the wide barrel-shaped spindle (Fig. 7 C). Thus, zw10 protein may be specifically associated with the KMTs, in accordance with its movement to the kinetochore region at anaphase onset. Moreover, although zw10 protein colocalizes with the leading edges of the chromosomes during anaphase at the resolution of light microscopy, we have no evidence that this site corresponds to the kinetochore per se. The resolution of these issues awaits further ultrastructural and biochemical studies.

The zw10 protein does not appear to correspond to any of the large number of proteins already known to inhabit the mitotic spindle or the kinetochore/centromere. Such proteins have been identified by several protocols: as microtubule-associated proteins (MAPs) based on their copurification with MTs (reviewed by Olmstead, 1986; Kellogg et al., 1989), on the basis of antisera obtained by immunization against nuclei (Frasch et al., 1986) or mitotic chromosome scaffolds (Compton et al., 1991), or as polypeptides recognized by antisera from various patients with autoimmune disease (CREST sera; Moroi et al., 1980). Recently, it has also been observed that cytoplasmic dynein is associated with kinetochores and the spindle (Pfarr et al., 1990; Steuer et al., 1990; Wordeman et al., 1991). To our knowledge, none of these or other components show the same pattern of cell cycle-specific localization as zw10 (see Brinkley, 1990; Pluta et al., 1990; Pankov et al., 1990; Earnshaw and Cooke, 1991).

The structure assumed by the zw10 antigen at metaphase is strongly evocative of the location of an antigen called spoke (Paddy and Chelsky, 1981). Anti-spoke antibodies stain KMTs, revealing a filamentous structure with a regular helical substructure, similar to that seen in Fig. 6, b and e and Fig. 7, A-C. However, we do not believe that zw10 represents the Drosophila homolog of spoke: unlike spoke, the metaphase structures identified by anti-zw10 antibodies extend through the chromosomal mass. Furthermore, spoke is not redistributed onto kinetochores at anaphase, and instead remains associated with KMTs (Paddy and Chelsky, 1991). It nonetheless remains possible that the zw10 metaphase structure is formed in association with a putative Drosophila spoke polypeptide.

The Role of zw10 in Mitotic Chromosome Segregation

Although the cytological evidence clearly shows that the zw10 product is required to ensure the accuracy of mitotic chromosome segregation, several unresolved issues preclude precise determination of its molecular function. Perhaps most importantly, we do not yet understand the significance of the high levels of PSCS observed in colchicine-treated zw10 neuroblasts. It is unclear whether this phenomenon monitors some indirect but nonetheless differential response of zw10 and wild-type brain cells to colchicine, or instead indicates differences in some inherent property of the centromeric attachment between sister chromatids. In addition, because zw10 mutations do not result in cell cycle arrest, zw10 activity cannot be unambiguously ascribed to a particular phase of the cell cycle. In spite of these gaps in our knowledge, the available cytological and immunocytochemical results nonetheless provides some clues to the possible roles played by the zw10 protein.

zw10 protein in the structure seen during metaphase that is roughly coincident with the spindle could be imagined to function in any of several ways. This protein could be of importance for spindle organization, for chromosome attachment or anchorage to the spindle, for ensuring the bipolar connection of sister kinetochores to opposite spindle poles, or for chromosomal movement during congression to the metaphase plate. However, preliminary three-dimensional observations in zw10 mutant brains and in embryos produced by homozygous mutant germline clones show no obvious defects in the structures of the metaphase spindle or of the metaphase plate (our own unpublished results). Of course, we cannot exclude the possibilities that subtle metaphase defects occur, or that zw10 activity during metaphase is required for subsequent anaphase movements. In addition, it should be remembered that one phenotypic effect of zw10 mutations, that of PSCS, presumably occurs during a metaphase-like state induced by colchicine (González et al., 1991).

We believe that hypotheses predicting an activity of zw10 protein at anaphase onset (rather than earlier at metaphase) are more compatible with the apparent normality of metaphase in mutants and with the extremely rapid transition between metaphase and anaphase locations of the zw10 antigen. The nature of the signals governing entry into anaphase remain quite mysterious (Murray et al., 1989), and little is understood of the crucial process that breaks the centromeric connection between sister chromatids at the beginning of anaphase (Murray and Szostak, 1985). We can thus only guess at the manner by which the movement of zw10 to the kinetochore region at anaphase onset could influence the accuracy of sister chromatid disjunction.

zw10 could be imagined to be a partially redundant component of a system positively required to activate sister chromatid separation or chromatid movement to the poles. Alternatively, zw10 protein could act as a feedback control rendering certain events at anaphase onset dependent upon the successful completion of earlier events. For example, zw10 might help ensure that anaphase will not begin if the spindle is not intact or if one or more chromosomes have not yet become properly aligned at the metaphase plate. It has been suggested that kinetochores unattached to the spindle may generate signals blocking anaphase onset (Zirkle, 1970; Ault and Nicklas, 1989; Rieder and Alexander, 1989; Bernat et al., 1991). Recently, "checkpoint" genes that apparently fulfill such a role have been found in yeast by Li and Murray (1991) and by Hoyt et al. (1991). Because we observe no obvious changes in cell cycle progression in zw10 mutants (Table II), delays of anaphase onset caused by zw10* activity would have to be quite short. In a different scenario, zw10* might render sister chromatid separation dependent upon M phase promoting factor (MPF) inactivation, which is normally a precondition for most anaphase events (Murray et al., 1989). Loss of such zw10*-mediated feedback inhibition could potentially explain the PSCS phenomenon observed in colchicine-treated mutant neuroblasts that retain high levels of MPF activity (Whitfield et al., 1990).

The unexpected distribution of the zw10 protein as a function of the cell cycle remains difficult to interpret in terms of molecular activities that explain the observed phenotypes. We nonetheless believe that these initial results are sufficiently intriguing that future studies of the zw10 protein and phenotype will provide unique insights into the function of the spindle and of the centromere/kinetochore.
We thank Maurizio Gatti, David Glover, and the members of their respective laboratories for their generosity, advice and assistance. We are indebted to Burke Judd and Abe Schalet for fly stocks, David Glover for anticientromosome antibodies, Janis Werner for embryo microinjection, Gary Chu for providing Northern blot strips, Ingrid Montuleone for supplying fly media, Diane LaPoint and Deborah Whiting for help with antibody production, and James Slattery, Carol Bayles, and Cathy Anderson for assistance with the confocal microscope at Cornell University.

This work was supported by National Institutes of Health (NIH) grant GM13995 and an NIH Fogarty Center Senior International Fellowship to M. L. Goldberg. B. C. Williams was supported by NIH training grant GM07617 to the Field of Genetics and Development at Cornell University.

Received for publication 3 February 1992 and in revised form 13 May 1992.

References


2:421-457.